Supporting Information

Supramolecular Encapsulation of Small-Ultra Red Fluorescent Proteins in Virus-Like Nanoparticles for Non-Invasive In Vivo Imaging Agents

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Instrumentation.

Dynamic-light scattering

Samples were measured using UV-Vis Malvern Panalytical Zetasizer Nano ZS. Each sample was loaded in a disposable microcuvette, measured at 25 °C with a 633 nm laser, 175 scattering angle, material refractive index of 1.51, and medium refractive index of 1.33.

UV-Vis

UV-Vis characterization was done using a Shimadzu UV-1601 PC UV-Vis-NIR Spectrophotometer using a 1.5 mL disposable cuvette. Sample concentrations were determined using a Biotek Synergy H4 hybrid reader or a Thermo Scientific NanoDrop 2000 Spectrophotometer.

Fluorescence Spectrophotometer

Measurements were performed using a Biotek Synergy H4 hybrid reader.

TEM

Transmission electron microscopy (TEM) was performed on a JEOL JEM-1400plus transmission electron microscope. Samples were prepared by incubating 5 μL of a 40 nM sample solution on a 300 mesh formvar-coated copper grid with 5 μL of 2% uranyl acetate for 60 s. Excess liquid was wicked away with Whatman (#1) filter paper before letting the grid dry in air. Images were taken with an accelerating voltage of 120 kV.

Gel Electrophoresis

1% agarose gels were run at 100 V for 30 mins with 1 × TBE running buffer. Samples were prepared in 100% glycerol. 10% SDS PAGE gels were run at 150 V for 75 min with SDS running buffer. Samples were prepared in SDS loading dye and ran using a Fisher BioReagents EZ-Run Prestained protein marker. Gels were first imaged by Biomolecular Imager-GE Typhoon FLA 9000 for Cy5 fluorescence. After, the gels were stained with Coomassie Brilliant Blue and imaged by BioRad ChemiDoc Gel Imager.

***In vivo* Fluorescence Imager**

Fluorescent animal imaging was taken with IVIS Lumina III (PerkinElmer, Waltham, MA, USA) at an excitation of 620 nm and emission at 670 nm with a 5 s exposure.

Supplementary Figures.

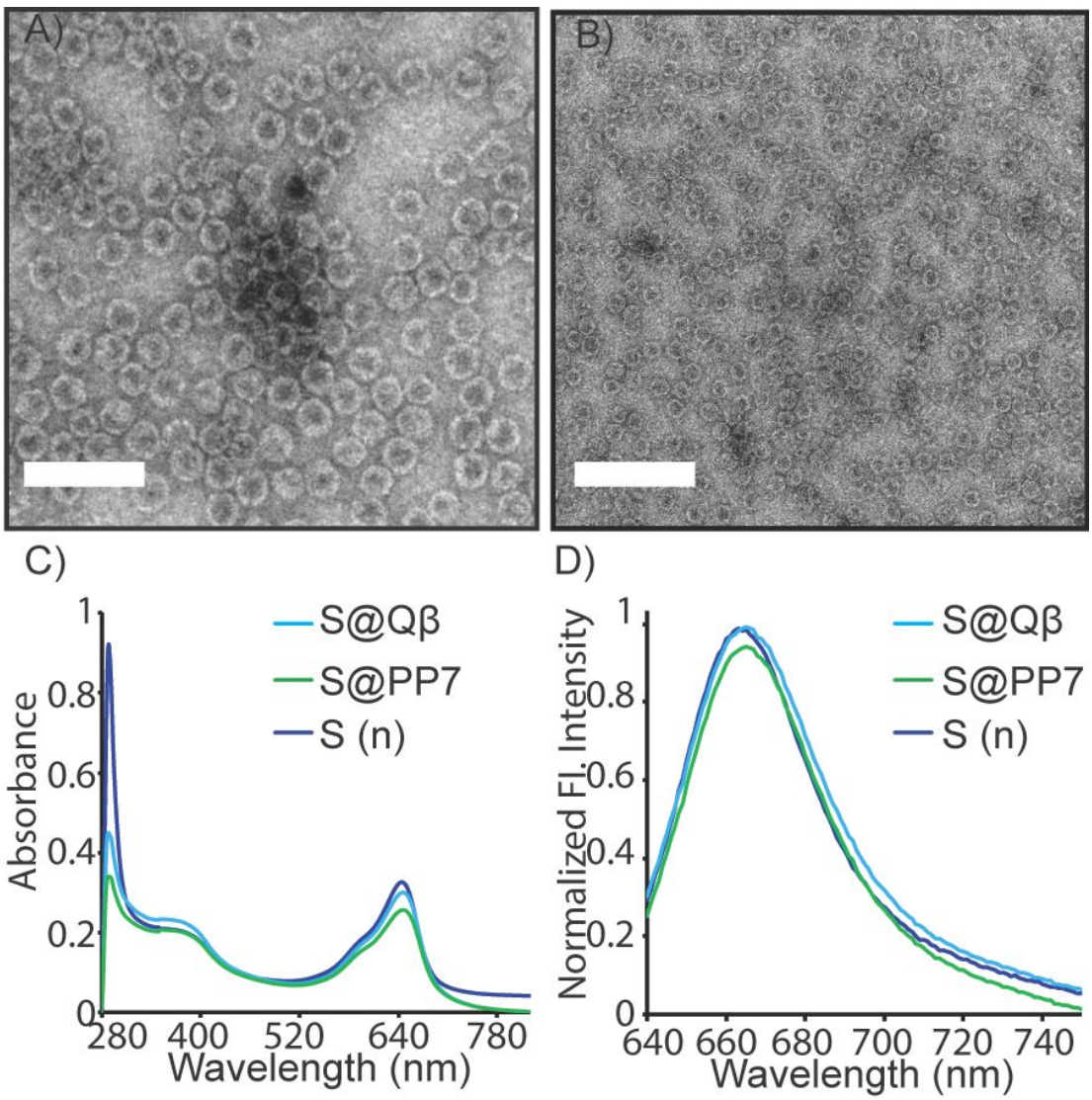

Figure S1 Native capsid characterization and spectral properties of fluorescent VLPs. A) TEM micrographs of Qβ and B) PP7. C) UV-Vis spectra of native and encapsulated smURFP. D) Emission spectra at λ_{max} 642 nm of native and encapsulated smURFP.

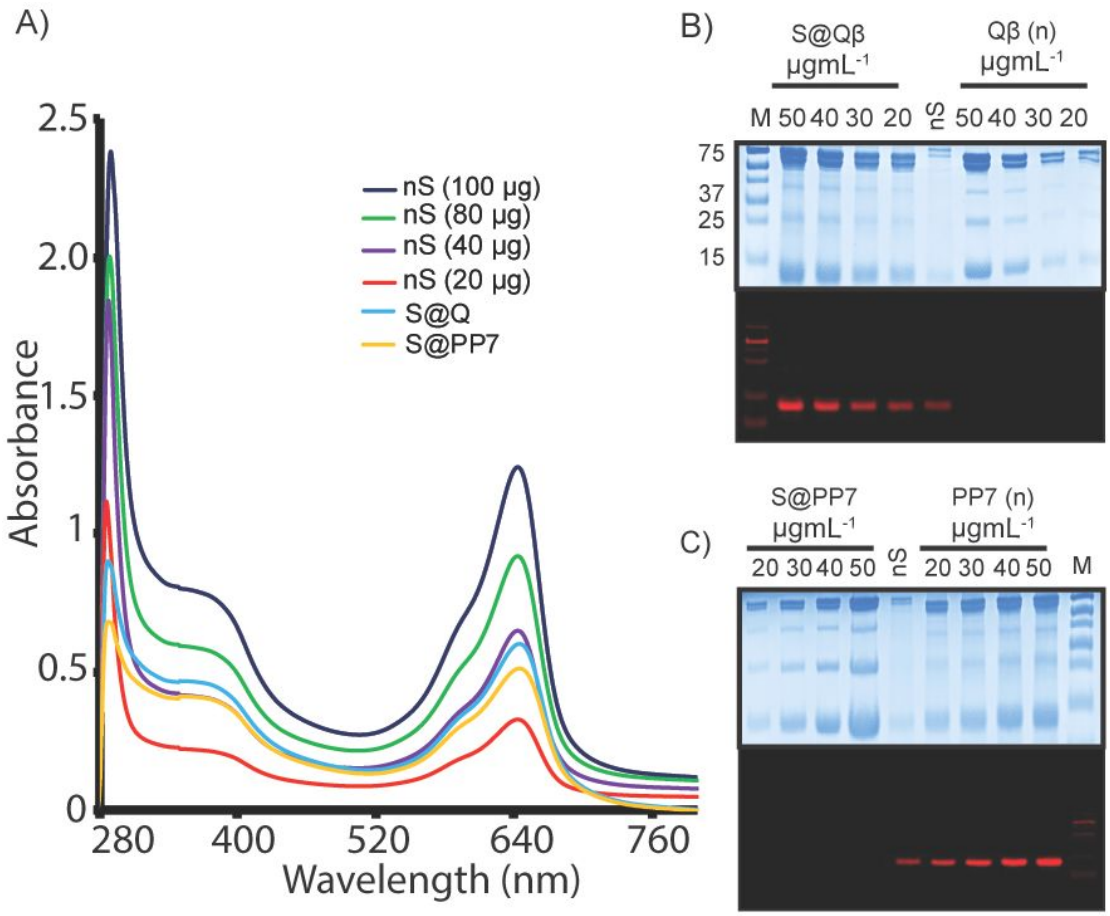

Figure S2. Quantification of smURFP proteins per VLP capsid. A) UV-Vis standard curve used for concentration determination of smURFP present in Qβ and PP7. B) 10% non-reducing SDS PAGE of native smURFP, native Qβ, and S@Qβ. C) 10% non-reduced SDS PAGE of native smURFP, native PP7, and S@PP7.

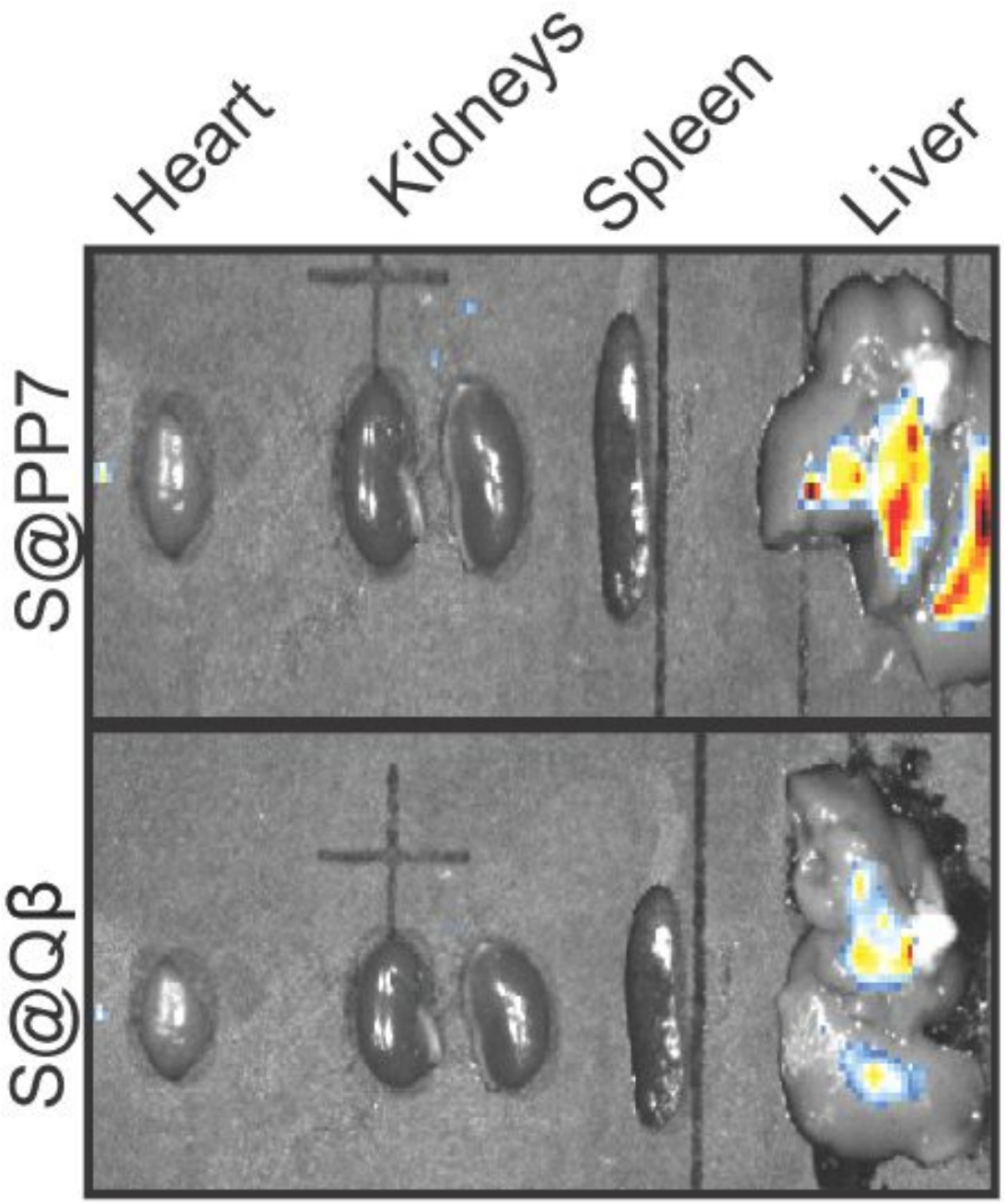

Figure S3. *Ex vivo* of intravenously injected S@VLPs. Organs extracted at 16 h time-point from mice injected intravenously with S@VLPs.